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A Study of the Interrelationship between the Triacylglycerol and Protein Components of Very-Low-Density Lipoproteins Using the Perfused Rat Liver

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1. High and low rates of very-low-density-lipoprotein triacylglycerol release from the perfused rat liver were achieved by using livers taken respectively from animals that had been given fructose for 48h or from animals that had been starved for 18h. 2. The higher rates of very-low-density-lipoprotein triacylglycerol release by the livers of the fructose-fed rats were associated with higher rates of very-low-density-lipoprotein protein release. 3. When the livers were perfused in the presence of [^{3}H]leucine, radioactivity was incorporated into the very-low-density-lipoprotein apoproteins. The higher rates of very-low-density-lipoprotein triacylglycerol and protein release by the livers of fructose-fed rats were associated with a greater total incorporation of radioactivity into those apoproteins that entered the running gel during polyacrylamide-gel electrophoresis. However, the distribution of radioactivity among the various apoproteins was not significantly changed by the dietary treatments used.

VLD lipoproteins* are the main carriers of triacylglycerol in the plasma in the post-absorptive state, and the main site of their synthesis is the liver (Hamilton, 1972).

Although the rates of triacylglycerol release from the liver are known to alter with changes in physiological and nutritional status (Nikkilä, 1969), little attention has been given to the question of whether such alterations involve corresponding changes in the rates of release of the proteins that are associated with the triacylglycerol in the VLD lipoproteins. Ruderman *et al.* (1968) have shown that the increase in the rate of triacylglycerol release by the perfused liver of the rat that occurs when the fatty acid concentration is raised in the perfusion fluid is accompanied by an increase in the rate of synthesis and release of the total VLD lipoprotein protein. However, other situations have not yet been examined from this point of view and, in particular, it is not known whether changes in triacylglycerol-release rates involve corresponding alterations in the rates of synthesis and release of the individual apoproteins of the VLD lipoproteins that are also known to be produced by the liver (Mahley *et al.*, 1970; Pottenger & Getz, 1971; Windmueller *et al.*, 1973; Noel & Rubinstein, 1974).

We report here the results of studies designed to investigate these questions further by using perfused livers from fructose-fed and starved rats respectively to achieve high and low triacylglycerol-release rates. The possibility of the metabolic modification of the VLD lipoproteins after their release has been minimized by the exclusion of plasma containing lipoproteins of higher density from the perfusion fluid.

* Abbreviation: VLD lipoprotein, very-low-density lipoprotein.

Materials and Methods

Animals

Two groups of male Wistar albino rats weighing 320–360g were used as liver donors. They had been maintained on Oxoid pasteurized diet 41B (Herbert Styles Ltd., Bewdley, Worcs., U.K.). The animals of one group were starved for 18h before use. The standard diet of the animals in the other group was replaced 48h before their use by fructose (100g/litre) in the drinking water. The average consumption of fructose over the 48h period was 20g per animal, representing a caloric intake adequate to meet the reported requirements (Mitchell, 1962).

Liver perfusions

The livers were perfused *in situ* by the method of Hems *et al.* (1966). The collecting vessel was replaced by one of smaller volume, thus decreasing to 50ml the minimum volume of perfusate required to maintain perfusion of the liver. The gas phase was O_2+CO_2 (95:5). Through the use of two identical perfusion systems in a single isothermal cabinet, livers from rats of each group were perfused simultaneously and the resultant samples handled in parallel.

The operative technique for the preparation of the liver was similar to that of Hems *et al.* (1966), except that the bile duct was left intact and heparin was excluded from all stages of the experiments. A flask containing 0.15M-NaCl was situated 15cm above the surgical table before the operation was

started. A siphon was established and clamped off in the flexible tubing extending from the flask to the portal-vein cannula. Immediately after this cannula had been tied into place, the clamp was removed and the NaCl solution was allowed to flow into the liver. At the same time, the thorax was opened and the right atrium was cut to allow the NaCl solution to flow freely through the liver. The thoracic vena cava was then cannulated, the abdominal vena cava was tied off and the preparation was connected into the perfusion system in which the oxygenated perfusion fluid was circulating; 1–2min elapsed from the time of cannulation of the portal vein to the time of connexion of the preparation into the perfusion system. Blood was cleared from all the lobes of the liver during this interval. The flow rate through the liver was 12–16ml/min, and the perfusion fluid pressure on the liver was never more than 20cm.

The initial perfusion fluid volume was 85ml. The perfusion fluid contained, in Krebs–Ringer bicarbonate buffer at pH7.4 (Krebs & Henseleit, 1932), 25% (v/v) washed erythrocytes, bovine serum albumin (30g/litre: Pentex, Miles Laboratories, Kankakee, Ill., U.S.A.), glucose (1g/litre) and 12mg of a mixture of amino acids (Morris, 1960) excluding leucine. In addition, that used for the livers from the fructose-fed rats contained fructose (0.2g/litre). This concentration is similar to that found in the portal veins of animals actively absorbing fructose (Topping & Mayes, 1972). The erythrocytes were collected from outdated human blood and washed on the day of the experiment (Hems *et al.*, 1966). The possibility of a reaction between human erythrocytes and rat blood in the liver was minimized by the procedure which cleared the liver of blood. To provide a fatty acid precursor for triacylglycerol production, a complex of oleic acid [Sigma (London) Chemical Co., Kingston-on-Thames, Surrey, U.K.] and albumin (Topping & Mayes, 1972) was infused continuously at a rate of 2ml (28μmol of oleic acid)/h so as to maintain a fatty acid concentration in the perfusion fluid of about 0.3μmol/ml. An initial priming dose of 2ml was given directly after the liver was connected into the system.

The livers were allowed to stabilize for 20min before the introduction into the perfusion system of 1.0ml of a 2% (v/v) ethanol solution containing 1mCi of L-[4,5-^{3}H]leucine (46Ci/mol: The Radiochemical Centre, Amersham, Bucks., U.K.). The [^{3}H]leucine was infused into the tubing connected to the portal-vein cannula and the time of completion of the infusion (1min) was designated as zero time. The perfusions were continued for an additional 4h.

Sampling

Samples of the perfusion fluid were taken from the tubing connected to the cannula leaving the liver. The sample sizes were 15ml at 1h and 10ml at 2h after the radioactive infusion. To avoid altering the composition of the perfusion fluid, the sample volume was not replaced. At 4h, the perfusion was discontinued and the remaining perfusion fluid was collected. All the samples were stored at 4°C after their collection.

Analytical techniques

After centrifugation to remove the erythrocytes, the VLD lipoproteins were isolated from the perfusion-fluid samples by centrifugation in polycarbonate tubes at 144000*g* and 4°C for 20h in a Beckman L1-40 preparative ultracentrifuge with a Beckman type 40 rotor. The samples were layered in the centrifuge tubes under 2ml of an NaCl solution of density 1.006 containing 0.1g of EDTA/litre and, after centrifugation, the VLD lipoprotein fraction was recovered by pipetting off the lipoprotein from the surface. After resuspension in the same NaCl solution, the VLD lipoprotein fraction was washed once by re-centrifugation under similar conditions.

The isolated VLD lipoprotein fractions were dialysed in purified dialysis tubing (Shore & Shore, 1973) for 48h at 4°C against 150vol. of 0.15M-NaCl containing 1mM-EDTA, pH8.6 (Kane, 1973), and 1mM-leucine. The solution was renewed after 24h. In a preliminary study, 250μCi of [^{3}H]leucine was added to 50ml of rat plasma and incubated at 37°C for 1h. The VLD lipoprotein fraction was then isolated and dialysed as described above. After the two periods of ultracentrifugation, the fraction contained 6% of the original radioactivity, but, after dialysis, this fell to 0.05%. Thus the procedures adopted for isolation and dialysis of the VLD lipoprotein were considered to be adequate to remove [^{3}H]leucine which was not incorporated into the VLD lipoprotein.

VLD lipoprotein and triacylglycerol were determined by the methods of Lowry *et al.* (1951) and Harwood *et al.* (1974) respectively and the concentrations of glucose and fructose in the perfusion fluid were determined by the methods of Krebs *et al.* (1963) and Roe (1934) respectively. Deoxycholate (50mg/ml) was used to clear the lipids in the VLD lipoprotein fraction before the protein determination. Mean values (±S.E.M.) are given in the text and the Figures. The values for triacylglycerol and protein release are corrected for sample removal.

Polyacrylamide-gel electrophoresis

The apoprotein subunits of the VLD lipoprotein were separated by gel electrophoresis in 7.5% (w/v) polyacrylamide by the method of Kane (1973) in the apparatus manufactured by Hoefer Scientific Instruments, San Francisco, Calif., U.S.A. The gels were

prepared in glass tubes 12.5cm long and 1.5cm internal diam. Between 150 and 250μl of the VLD lipoprotein samples, containing between 5 and 50μg of protein and at least 10^5 d.p.m., was used, and 50μl of Bromophenol Blue (10mg/litre; May and Baker, Dagenham, Essex, U.K.) was added to the upper tank buffer to show the progress of the buffer front. After electrophoresis, the gels were fixed, stained with Coomassie Blue (Sigma), and finally destained in a continuous-flow apparatus (Hoefer Scientific Instruments). All these procedures were carried out by the method of Fishbein (1972). The gels were then scanned at 565nm in a Unicam SP.1800 densitometer, and sliced into 1mm slices with a gel slicer (model 190; Bio-Rad Laboratories, Bromley, Kent, U.K.) for the radioactivity measurements.

The method of Kane (1973) uses 1,1,3,3-tetramethylurea to delipidate the lipoproteins and to liberate the apoproteins. The treatment with tetramethylurea may be carried out either before or after the application of the lipoprotein to the spacer gel. However, a fraction of the VLD lipoprotein apoproteins is insoluble in tetramethylurea (Kane, 1973), and, in our experience, it associates with the liberated lipid so that, on centrifugation, it is present mainly in the floating lipid layer. When the treatment with tetramethylurea is carried out before application to the spacer gel, the aqueous phase is difficult to transfer to the gel quantitatively. For these reasons, in the present experiments the treatment was carried out as a routine on the gel surface. However, an additional VLD lipoprotein sample was incubated as a routine with tetramethylurea in a test tube and, after centrifugation, a portion of the aqueous phase was taken for radioactivity determination. If, as has been reported (Kane, 1973), only the apoproteins soluble in tetramethylurea enter the running gel, the radioactivity in this aqueous phase should equal the radioactivity recovered from the running gel. In fact, in the present experiments, the latter amounted to 95$\pm$8% of the former.

Using VLD lipoproteins from human serum, Kane (1973) measured the protein contents of the native lipoprotein fraction and of the tetramethylurea aqueous phase. Thus, by difference, he was able to determine the protein content of the tetramethylurea-insoluble material. However, a fivefold dilution of the tetramethylurea aqueous phase was necessary to overcome interference by tetramethylurea with the determination of protein. Although it would have been desirable to know the protein content of the material soluble and insoluble in tetramethylurea in our experiments, the concentration of protein in the fractions was not sufficient for such a fivefold dilution.

All the reagents used for the polyacrylamide-gel electrophoresis were obtained from British Drug Houses, Poole, Dorset, U.K., except for the tetramethylurea (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.) and the potassium persulphate (Hopkin and Williams, Chadwell Heath, Essex, U.K.). The urea (AnalaR grade) was freed from cyanate by passing a saturated solution through an Amberlite MB-3 column. After recrystallization, it was dissolved in the reactant solutions within the hour preceding the pouring of the gels.

Determination of radioactivity

The amounts of radioactivity in portions of the intact VLD lipoprotein fractions (50μl) and of the aqueous phase after delipidation with tetramethylurea (100μl) were measured by adding them directly to 10ml of a scintillant solution containing 7g of 2,5-diphenyloxazole, 600mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene and 150g of naphthalene in 500ml of 2-ethoxyethanol and 1litre of toluene (White, 1968), all these materials being purchased from Fisons, Loughborough, Leics., U.K. The amounts of radioactivity in the gel slices obtained after polyacrylamide-gel electrophoresis were measured after treatment with 30% (v/v) H_2O_2 (200μl) in the counting vials (Windmueller *et al.*, 1973). The vials were capped and heated overnight at 60°C. Then 10μl of catalase was added, to prevent quenching by any O_2 present, followed by 10ml of the scintillant solution. All the samples were counted three times for 5min in a Beckman LS230 liquid-scintillation spectrometer and the mean counts were corrected for quenching by the channel-ratio method.

Results

The data presented were obtained during three perfusions of livers from starved rats and three perfusions of livers from fructose-fed rats. On consecutive days and at 09:30h, a liver from a rat of one group was perfused simultaneously with a liver from a rat of the other group. The lipoprotein samples from all of the experiments were analysed at the same time. Several similar experiments have been completed and the results have confirmed those presented here.

Glucose and fructose concentrations in the perfusion fluid

During the liver perfusions, the glucose concentration in the perfusion fluid rose from its initial value of 1mg/ml. After 4h it reached 2.6$\pm$0.1mg/ml in the experiments with livers from starved rats and 6.1$\pm$0.1mg/ml in the experiments with livers from fructose-fed rats. The higher concentrations in the latter may indicate a greater degree of glycogenolysis (Elliott *et al.*, 1971).

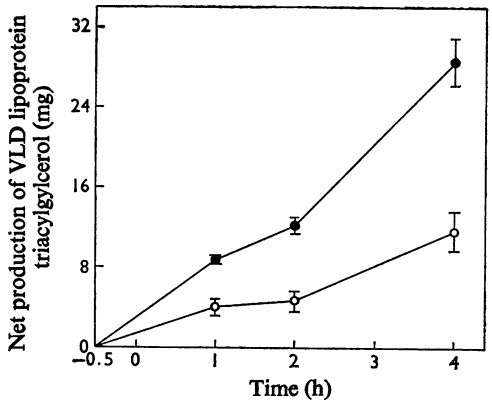

Fig. 1. *Secretion of VLD lipoprotein triacylglycerol by perfused rat livers*

VLD lipoproteins were isolated from samples of the perfusion fluid taken at the times indicated and analysed for triacylglycerol. The donor rats were starved (○) or fructose-fed (●). Three experiments were carried out with each group and the mean values at each time-interval are given. The bars indicate the S.E.M. Zero time was the time of infusion of radioactive leucine into the perfusion fluid (see the Materials and Methods section).

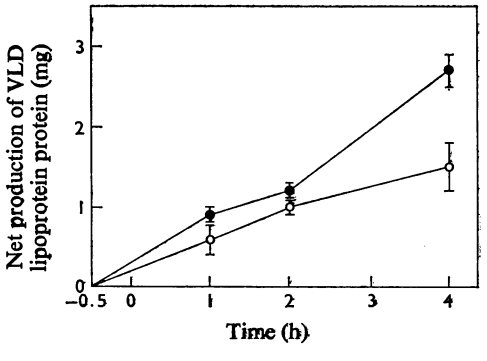

Fig. 2. *Secretion of VLD lipoprotein by perfused rat livers*

VLD lipoproteins were isolated from samples of the perfusion fluid taken at the times indicated, and analysed for protein. The donor rats were starved (○) or fructose-fed (●). Three experiments were carried out with each group and the mean values at each time-interval are given. The bars indicate the S.E.M. Zero time was the time of infusion of radioactive leucine into the perfusion fluid (see the Materials and Methods section).

Fructose was present initially in the perfusion fluid used for the livers from fructose-fed rats at a concentration of 0.2mg/ml. In agreement with the findings of others that fructose is removed rapidly by the liver (Woods *et al.*, 1970: Topping & Mayes, 1972), this was completely removed during the 4h perfusion period.

Table 1. *Triacylglycerol/protein ratio in liver perfusate VLD lipoproteins*

Three livers from starved and three livers from fructose-fed rats were perfused as described in the text. Triacylglycerol and protein were measured in VLD lipoprotein isolated from the perfusion fluid at the times shown and the mean (±S.E.M.) values for the triacylglycerol/protein ratio are given.

Time (h)	Triacylglycerol/protein ratios: Livers from starved rats	Livers from fructose-fed rats
1	9.5±2.8	9.6±1.0
2	4.7±0.8	10.3±1.8
4	7.6±1.1	10.6±1.9

Triacylglycerol and protein concentrations in VLD lipoproteins in the perfusion fluid

The results in Figs. 1 and 2 show that the rates of secretion of both VLD lipoprotein triacylglycerol and protein by the livers of fructose-fed rats were greater than by the livers of starved rats. Over a 4h perfusion period, the livers from fructose-fed rats secreted about 2.5 times the amount of VLD lipoprotein triacylglycerol and almost two times the amount of VLD lipoprotein than did the livers from starved rats.

There was no lag in the release of either VLD lipoprotein triacylglycerol or protein in either group, in agreement with the results of Topping & Mayes (1972) and Noel & Rubinstein (1974). The absolute rate of triacylglycerol release was similar to that reported by Ruderman *et al.* (1968), who used a similar perfusion fluid. No protein was recovered in the VLD lipoprotein fraction at the beginning of the perfusion period, indicating that the procedure for its isolation eliminated carry-over of albumin from the perfusion fluid.

The ratios of triacylglycerol to protein in the VLD lipoprotein secreted by the livers of the starved and fructose-fed animals are shown in Table 1. Although the ratio for livers from starved animals varies at different perfusion times, it is significantly ($P<0.05$) lower than that for the livers from fructose-fed animals after perfusion for 2h and 4h.

Incorporation of [^{3}H]leucine into VLD lipoprotein apoproteins

In the presence of [^{3}H]leucine in the perfusion fluid, radioactivity was incorporated into the isolated VLD lipoprotein fraction in perfusions carried out with livers from both fructose-fed and starved

rats. The extent of incorporation in the former after perfusion for 4h was almost twice that in the latter. Thus the difference between the groups was similar to that observed for total VLD lipoprotein, the mean specific activities (d.p.m./μg of VLD lipoprotein protein) after 4h of perfusion being respectively 5700±500 and 5300±100. These data could be interpreted as suggesting that, in agreement with the findings of Ruderman *et al.* (1968), a correspondence was maintained between VLD lipoprotein synthesis and release when the rate of VLD lipoprotein triacylglycerol release was altered. However, if any of the [^{3}H]leucine was incorporated into the VLD lipoprotein lipid during the perfusion, this would not be distinguished from incorporation into the VLD lipoprotein protein under the conditions of analysis used here.

Of special interest in the present study was the possibility that incorporation of [^{3}H]leucine into the individual apoproteins that are known to have specific functions in the metabolism of the VLD lipoprotein (Fredrickson *et al.*, 1972; Havel *et al.*, 1973; Krauss *et al.*, 1973) was differentially affected when the rate of VLD lipoprotein triacylglycerol release by the liver was altered. Experiments to test this were carried out with VLD lipoprotein isolated from samples of perfusion fluid taken at 4h and delipidated with tetramethylurea by the method of Kane (1973).

Of the total radioactivity incorporated into the VLD lipoprotein fraction in these experiments, a large percentage (77±6% and 82±4% in the fructose-fed and starved groups of animals respectively) was insoluble in the tetramethylurea (see the Materials and Methods section). The main protein in this fraction is likely to correspond to the apoprotein B of human serum VLD lipoprotein (Kane, 1973; Goldstein *et al.*, 1974), and this protein is already known to be labelled when rat livers are perfused with radioactive amino acids (Windmueller *et al.*, 1973; Noel & Rubinstein, 1974). In the present study, no attempt was made to assess the possible contribution of radioactive lipid to the total radioactivity in the tetramethylurea-insoluble fraction and, for this reason, our absolute values must be treated with reserve. However, it is noteworthy that the percentages were similar in livers from rats showing different rates of triacylglycerol release.

The VLD lipoprotein apoproteins of particular significance in the present study were those that entered the running gel on polyacrylamide-gel electrophoresis (Fredrickson *et al.*, 1972). A representative densitometer tracing of such a running gel, stained with Coomassie Blue, is shown in Fig. 3. The pattern of distribution of the apoproteins is similar to that which has previously been described in studies with VLD lipoproteins isolated from rat plasma and from rat liver perfusion systems in which delipidation was carried out with organic solvents (Koga *et al.*, 1969, 1971; Bersot *et al.*, 1970; Mahley *et al.*, 1970; Rubenstein & Rubinstein, 1972; Windmueller *et al.*, 1973; Noel & Rubinstein, 1974). However, there is somewhat greater resolution of the polypeptides of higher mobility (zone B, Fig. 3) and the densitometer scan shows five to six bands in this region that were also visible to the naked eye.

The results in Table 2 show that, despite the differences in the triacylglycerol release rates, there were no significant differences between the livers from the starved and the fructose-fed rats in the distribution of radioactivity between the apoproteins of zones A and B in the running gel. Although about 20% of the radioactivity that entered the running gel was in neither zone A nor zone B, this was also found with both groups of livers to be distributed roughly evenly on either side of zone A.

The results reported in detail in Table 2 are those obtained by analysis of VLD lipoprotein fractions isolated from the perfusion fluid samples taken at 4h. Because of the increased protein and ^{3}H content of the VLD lipoproteins at this time, smaller volumes were required for electrophoresis and this led to greater resolution of the apoproteins. However, in separate experiments analyses were carried out on

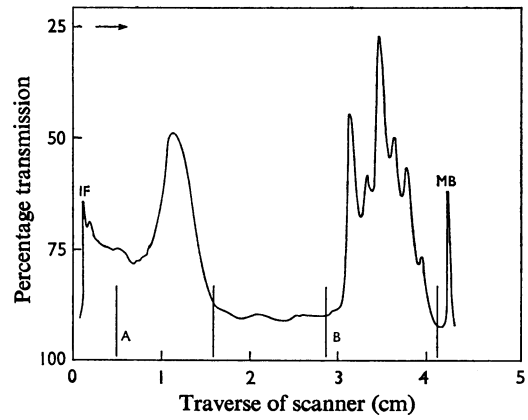

Fig. 3. *Densitometer tracing of stained VLD lipoprotein apoproteins after polyacrylamide-gel electrophoresis*

A VLD lipoprotein sample, isolated from the perfusion fluid of a liver taken from a fructose-fed rat, was submitted to treatment with tetramethylurea and polyacrylamide-gel electrophoresis (see the Materials and Methods section). After electrophoresis, the gel was fixed, stained with Coomassie Blue, destained and finally scanned at 565nm. IF denotes the interface between the spacer gel and the running gel. The arrow shows the direction of migration. MB is the Bromophenol Blue marker band indicating the progress of the buffer front. The zones designated A and B contain the major portion of staining material in the running gel and each subsequently yielded between eight and ten gel slices for radioactivity measurements (see the text.).

Table 2. *Distribution of radioactivity in VLD-lipoprotein apoproteins after polyacrylamide-gel electrophoresis*

Three livers from starved rats and three livers from fructose-fed rats were perfused as described in the text. The perfusing fluid contained [^{3}H]leucine. After 4h, VLD lipoproteins were isolated from the perfusate, and polyacrylamide-gel electrophoresis of the apoproteins was carried out by the method of Kane (1973). The radioactivity in zones A and B (see Fig. 3 and the text) was measured as described in the Materials and Methods section. Mean (±S.E.M.) values are given.

Region of running gel	% of running-gel radioactivity	
	Livers from starved rats	Livers from fructose-fed rats
Zone A	20.8±4.9	24.7±1.2
Zone B	58.0±4.4	55.4±1.2
Sum	78.8±0.6	80.1±0.2

VLD lipoproteins isolated from samples of perfusion fluid collected at 1 and 2h. In these no differences from the 4h samples were evident in the apoprotein pattern, in the percentage of the radioactivity that entered the running gel, or in the percentage of radioactivity in corresponding slices of the running gel. In all the experiments, the radioactivity in zones A and B was generally proportional to the intensity of staining with Coomassie Blue and the radioactivity of each of the bands in zone B generally corresponded to the intensity of their staining. Noel & Rubinstein (1974) have also reported an overall correspondence between the intensity of protein staining and the distribution of radioactivity in the VLD lipoprotein apoproteins separated by polyacrylamide-gel electrophoresis.

Discussion

Under the conditions in which the rate of VLD lipoprotein triacylglycerol secretion by the liver is increased, the rate of secretion of the protein moiety of the VLD lipoprotein might be expected to remain constant, to increase in correspondence with the rise in the triacylglycerol-secretion rate or to alter independently of the change in the rate of triacylglycerol secretion. The studies of Ruderman *et al.* (1968) have shown that increases in VLD lipoprotein triacylglycerol-secretion rate by the rat liver that are brought about by a rise in the fatty acid concentration in the perfusion fluid are associated with increased VLD lipoprotein protein release and with some increase in the VLD lipoprotein triacylglycerol/protein ratio. The present investigation has extended their work to situations where different rates of triacylglycerol release have been achieved by using livers from rats in different nutritional states, rather than by changes in the availability of fatty acids. Again an increase in the rate of VLD lipoprotein triacylglycerol release was accompanied by an increase in the rate of VLD lipoprotein protein secretion and by a rise in the VLD lipoprotein triacylglycerol/protein ratio.

The present findings are also in agreement with those of Ruderman *et al.* (1968) in showing that increases in VLD lipoprotein triacylglycerol-release rates are accompanied by increases in the rates of VLD lipoprotein protein synthesis by the liver. They are also consistent in this respect with the whole-animal studies of Schiff *et al.* (1971), using rats on sucrose-rich diets. However, we have obtained no evidence to suggest that such increases in the rates of VLD lipoprotein protein synthesis involve alterations in the relative rates of synthesis of the individual VLD lipoprotein apoproteins. Thus we were able to detect neither changes in the relative amounts of the different apoproteins nor in the distribution of [^{3}H]leucine incorporation into them. Differences in apoprotein distribution in the plasma VLD lipoproteins have been reported in sucrose-fed rats (Roheim *et al.*, 1973), and in unpublished studies we have observed similar differences in fructose-fed animals. Such changes could occur as a result of the metabolism of VLD lipoprotein after its release from the liver, however (Hamilton, 1972; Bilheimer *et al.*, 1972; Eisenberg *et al.*, 1972, 1973; Rubenstein & Rubinstein, 1972, 1973; Eisenberg & Rachmilewitz, 1973). It is also possible that more prolonged dietary changes than those used here would lead to alterations in the rates of hepatic synthesis of the individual VLD lipoprotein apoproteins.

Plasma proteins were omitted from the perfusion fluid used in the present study to prevent any exchange of newly synthesized VLD lipoprotein apoproteins with their counterparts which exist in the plasma high-density lipoproteins. Although we did not investigate whether classes of lipoproteins other than VLD lipoproteins were released during the liver perfusions, recent studies suggest that low-density lipoproteins are not synthesized and released as such (Hamilton, 1972; Noel & Rubinstein, 1974) and that the apoproteins of the high-density lipoproteins which are synthesized and released as such are mainly those present to only a small extent in the VLD lipoproteins (Rubenstein & Rubinstein, 1973; Noel & Rubinstein, 1974). Loss of newly synthesized VLD lipoprotein apoproteins during the perfusion by exchange with their counterparts in the high-density lipoprotein fraction is likely, therefore, to have been small under the conditions of the present experiments.

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References

Bersot, T. P., Brown, W. V., Levy, R. I., Windmueller, H. G., Fredrickson, D. S. & LeQuire, V. S. (1970) *Biochemistry* **9**, 3427–3433

Bilheimer, D. W., Eisenberg, S. & Levy, R. I. (1972) *Biochim. Biophys. Acta* **260**, 212–221

Eisenberg, S. & Rachmilewitz, D. (1973) *Biochim. Biophys. Acta* **326**, 391–405

Eisenberg, S., Bilheimer, D. W. & Levy, R. I. (1972) *Biochim. Biophys. Acta* **280**, 94–104

Eisenberg, S., Bilheimer, D. W., Levy, R. I. & Lindgren, F. W. (1973) *Biochim. Biophys. Acta* **326**, 361–377

Elliott, J., Hems, D. A. & Beloff-Chain, A. (1971) *Biochem. J.* **125**, 773–780

Fishbein, W. N. (1972) *Anal. Biochem.* **46**, 388–401

Fredrickson, D. S., Lux, S. E. & Herbert, P. N. (1972) *Adv. Exp. Med. Biol.* **26**, 25–56

Goldstein, S., Pinon, J. C., Bridoux, A. M., Kha Tu, H. & Loudat, Ph. (1974) *FEBS Lett.* **41**, 335–338

Hamilton, R. L. (1972) *Adv. Exp. Med. Biol.* **26**, 7–24

Harwood, J. L., Riley, S. E. & Robinson, D. S. (1974) *Biochim. Biophys. Acta* **337**, 225–238

Havel, R. J., Fielding, C. J., Olivecrona, T., Shore, V. G., Fielding, P. E. & Egelrud, T. (1973) *Biochemistry* **12**, 1828–1833

Hems, R., Ross, B. D., Berry, M. N. & Krebs, H. A. (1966) *Biochem. J.* **101**, 284–292

Kane, J. P. (1973) *Anal. Biochem.* **53**, 350–364

Koga, S., Horwitz, D. L. & Scanu, A. M. (1969) *J. Lipid Res.* **10**, 577–588

Koga, S., Bolis, L. & Scanu, A. M. (1971) *Biochim. Biophys. Acta* **236**, 416–430

Krauss, R. M., Herbert, P. N., Levy, R. I. & Fredrickson, D. S. (1973) *Circ. Res.* **33**, 403–411

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–36

Krebs, H. A., Bennett, D. A. H., de Gasquet, P., Gascoyne, T. & Yoshida, T. (1963) *Biochem. J.* **86**, 22–27

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Mahley, R. W., Bersot, T. P., LeQuire, V. S., Levy, R. I., Windmueller, H. G. & Brown, W. V. (1970) *Science* **168**, 380–382

Mitchell, H. H. (1962) *Comparative Nutrition of Man and Domestic Animals*, vol. 1, p. 22, Academic Press, London and New York

Morris, B. (1960) *Aust. J. Exp. Biol.* **38**, 99–110

Nikkilä, E. A. (1969) *Adv. Lipid Res.* **7**, 63–124

Noel, S. & Rubinstein, D. (1974) *J. Lipid Res.* **15**, 301–308

Pottenger, L. A. & Gretz, G. S. (1971) *J. Lipid Res.* **12**, 450–459

Roe, J. H. (1934) *J. Biol. Chem.* **107**, 15–22

Roheim, P. S., Edelstein, D. & Vega, G. (1973) *Circulation* **48**, IV-208 (Abstract)

Rubenstein, B. & Rubinstein, D. (1972) *J. Lipid Res.* **13**, 317–324

Rubenstein, B. & Rubinstein, D. (1973) *J. Lipid Res.* **14**, 357–363

Ruderman, N. B., Richards, K. C., Valles de Bourges, V. & Jones, A. L. (1968) *J. Lipid Res.* **9**, 613–619

Schiff, T. S., Roheim, P. S. & Eder, H. A. (1971) *J. Lipid Res.* **12**, 596–603

Shore, V. G. & Shore, B. (1973) *Biochemistry* **12**, 502–507

Topping, D. L. & Mayes, P. A. (1972) *Biochem. J.* **126**, 295–311

White, D. R. (1968) *Int. J. Appl. Radiat. Isot.* **19**, 49–61

Windmueller, H. G., Herbert, P. N. & Levy, R. I. (1973) *J. Lipid Res.* **14**, 215–223

Woods, H. F., Eggleston, L. V. & Krebs, H. A. (1970) *Biochem. J.* **119**, 501–510